FURTHER STUDIES ON THE VIRULENT SALT SOLUTION USED IN THE PRODUCTION OF HOG-CHOLERA SERUM *

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No accurate method of standardizing hog-cholera virus has been found. Certain strains of it, however, can usually be developed to such a point as to yield fairly uniform results. In the production of hog-cholera serum at this station, the original (Ames, Iowa, 1908) Dorset-Niles strain of virus is used, which, when injected intramuscularly in doses of from 3 to 5 c.c., almost invariably produces typical symptoms of hog cholera and death in susceptible pigs, averaging 100 lb. each in weight, in from 6 to 7 days. When kept at this virulence, it has never failed, by subcutaneous methods, to produce potent serum. In studying the nature of virulent salt solution, I have accepted this known strain of virulent blood as a standard of comparison, and have endeavored accurately to compare virulent salt solution with virulent blood from the same virus pigs.

Graham and Himmelberger[1] found virulent salt solution efficient in hyperimmunization by the intravenous method when mixed with virulent blood. However, their work gives no information as to the virulence of salt solution, since the two viruses were used together and in each case 7 c.c. of the mixture to the pound of body weight.

Craig[2] found that 10 pigs inoculated intramuscularly with 2 c.c. each of virulent salt solution lived an average period of 13 days. Nine pigs injected with 2-c.c. doses of virulent blood from the same source lived an average period of 9 days. No mention is made of how long the salt solution remained in the abdominal cavities of the virus pigs nor of the amount injected into them. From these tests it appears that the salt solution used was not as virulent as virulent blood.

Such tests, by direct subcutaneous or intramuscular inoculation, do not appear to be an accurate means of standardizing virulent salt

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[1] Jour. Infect. Dis., 1916, 18, p. 118.
[2] Bull. Purdue Univ. Agr. Exper. Sta. No. 173, 1914, p. 446.

solution, as the virulent salt solution is absorbed more readily and more completely than the virulent blood. Direct intravenous inoculations perhaps would give more accurate results. It also seems reasonable that hog-cholera virus could be accurately tested by using it to hyperimmunize by the intravenous method and then testing the serum secured against a known strain of virus.

In my previous work[3] I found that 6-hour 25-c.c. virulent salt solution (salt solution injected into the abdominal cavity of virus pigs at the rate of 25 c.c. to the pound of body weight and allowed to remain for 6 hours) by the subcutaneous methods would uniformly produce serum of high potency. In the hyperimmunization of several hundred pigs by the subcutaneous methods with mixtures usually of about equal parts of virulent blood and virulent salt solution, I have produced a very potent serum in every case. In so far as the production of hog-cholera serum by the subcutaneous methods is concerned, we may accept virulent salt solution as equal to virulent blood as a virus for hyperimmunization. However, this does not mean that 6-hour 25-c.c. virulent salt solution is as virulent as virulent blood. The absorption of any liquid injected subcutaneously or intramuscularly varies according to the density as well as the bacterial and chemical contents of the liquid. The use of virulent salt solution has reduced to a minimum the abscesses that follow subcutaneous injections of straight virulent blood. In many cases these local abscesses no doubt mean a loss of one-half the virulent blood injected, the blood becoming encysted at the point of inoculation and so shut off from the absorptive system. Therefore, it appears that if virulent salt solution were only one-half as virulent as virulent blood, it might produce serum of potency equal to that produced by virulent blood by the subcutaneous methods, on account of its being more completely absorbed. When used together with virulent blood, the efficiency of the latter is increased because, the density being lowered, the degree of absorption is increased.

Before making further biologic tests of virulent salt solution it was thought advisable to learn something of its chemical and bacteriologic contents as compared with those of virulent blood. With the assistance of Mr. Herman Waagbo, I have made analyses of the two viruses from 25 virus pigs, the results of which are given in Table 1.

The data show that virulent salt solution contains very small percentages of fibrin and protein as compared with virulent blood and a much smaller number of colonies of B. cholera-suis, altho this organ-

[3] Jour. Infect. Dis., 1913, 12, p. 335.

TABLE 1

COMPARATIVE ANALYSIS OF VIRULENT SALT SOLUTION AND VIRULENT BLOOD FROM THE SAME PIGS

Number of Pigs	Rate of Injection, c.c. per lb.	Time in Abdominal Cavity hr.	Percentage Recovered	Average Percentage of Fibrin		Average Percentage of Protein		Colonies B. Cholera-Suis per c.c.	
				Virulent Salt Solution	Virulent Blood	Virulent Salt Solution	Virulent Blood	Virulent Salt Solution	Virulent Blood
5	25	4	68.6	.058	1.09	.45	15.55	1275	15,350
5	25	5	68.7	.041	1.49	.59	17.41	41	63,139
5	25	6	61.4	.067	0.62	.99	17.75	3	6,468
5	25	7	48.0	.077	1.16	.65	16.18	92	223,330
5	25	8	53.0	.082	1.01	.99	17.68	538	2,343

ism was uniformly present. The percentages of fibrin and protein in the salt solution increased with the time it remained in the abdominal cavity.

Since the potency of hog-cholera serum depends largely on the virulence of the virus used in its production, it seemed that an accurate comparison of virulent salt solution and virulent blood through the hyperimmune, using the intravenous method, would give us some knowledge of the virulence of salt solution. With this in view I drew 6-hour 25-c.c. virulent salt solution and virulent blood from the same virus pigs and used these viruses as "straight virulent salt solution" and "straight virulent blood" in hyperimmunizing 6 pigs by the intravenous method. Three were injected with virulent salt solution at the rate of 6 c.c. to the pound of body weight, and 3 at the same rate with virulent blood. One of the hyperimmunes receiving blood died soon after the injection. The other two were carried through the experi-

TABLE 2

REROD OF TEST

Pig	Weight, lb.	Serum Injected c.c.		Virus Injected c.c.		Results
953	80	25	Virulent-salt-solution hyperimmunes	2	Stock No. 577	Died within 10 days; typical cholera lesions
954	90	20		2		Died within 8 days; typical cholera lesions
955	75	15		2		Died within 8 days; typical cholera lesions
956	90	25	Virulent-blood hyperimmunes	2		Lived
957	100	20		2		Lived
958	75	15		2		Lived
959	95	None		2		Killed on 7th day; used in serum production
960	90	None		2		Killed on 7th day; used in serum production

ment together with the three that had received the salt solution. The sera from the latter were mixed together and likewise those from the two hyperimmunized with virulent blood. The resultant mixed sera were tested on 8 susceptible pigs as indicated in Table 2.

The results of this test indicate that virulent salt solution is not nearly so virulent as virulent blood, and that while it is efficient in hyperimmunizing by the subcutaneous method, it would no doubt be impractical in the intravenous method because of the difficulty of injecting enough of it intravenously to produce very potent serum. Even if it is used in mixture with virulent blood, the amount of the mixture required to produce good serum would be unusually high.